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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 :
C12N 15/12, C07K 14/705, C12N 5/10, C12Q 1/68, G01N 33/68

A1

(11) International Publication Number: **WO 98/38301**

(43) International Publication Date: 3 September 1998 (03.09.98)

(21) International Application Number: PCT/CA98/00173

(22) International Filing Date: 27 February 1998 (27.02.98)

(30) Priority Data:

60/039,204	28 February 1997 (28.02.97)	US
09/030,482	25 February 1998 (25.02.98)	US

(81) Designated States: CA, JP, European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).

Published
With international search report.
Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.

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(54) Title: HUMAN CALCIUM CHANNELS ALFA1 SUBUNITS AND RELATED PROBES, CELL LINES AND METHODS

(57) Abstract

Partial sequences for a novel mammalian (human and rat sequences identified) calcium channel subunit which we have labeled as the α_{1I} subunit, and an additional novel human calcium channel which we have labeled as the α_{1H} subunit are provided. Knowledge of the sequence of these two calcium channels permits the localization and recovery of the complete sequence from human cells, and the development of cell lines which express the novel calcium channels of the invention. These cells may be used for identifying compounds capable of acting as agonists or antagonists to the calcium channels.

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HUMAN CALCIUM CHANNELS ALFA1 SUBUNITS AND RELATED PROBES. CELL LINES AND METHODS

DESCRIPTION

TECHNICAL FIELD

The present invention relates to novel human calcium channel compositions, and to the expression of these compositions in cell lines for use in evaluating calcium channel function.

BACKGROUND OF THE INVENTION

The rapid entry of calcium into cells is mediated by a class of proteins called voltage-gated calcium channels. Calcium channels are a heterogeneous class of molecules that respond to depolarization by opening a calcium-selective pore through the plasma membrane. The entry of calcium into cells mediates a wide variety of cellular and physiological responses including excitation-contraction coupling, hormone secretion and gene expression. In neurons, calcium entry directly affects membrane potential and contributes to electrical properties such as excitability, repetitive firing patterns and pacemaker activity. Miller, R.J. (1987) Multiple calcium channels and neuronal function. Science 235:46-52. Calcium entry further affects neuronal functions by directly regulating calcium-dependent ion channels and modulating the activity of calcium-dependent enzymes such as protein kinase C and calmodulin-dependent protein kinase II. An increase in calcium concentration at the presynaptic nerve terminal triggers the release of neurotransmitter. Calcium entry also plays a role in neurite outgrowth and growth cone migration in developing neurons and has been implicated in long-term changes in neuronal activity. In addition to the variety of normal physiological functions mediated by calcium channels, they are also implicated in a number of human disorders. Recently, mutations identified in human and mouse calcium channel genes have been found to account for several disorders including, familial hemiplegic migraine, episodic ataxia type 2, cerebellar ataxia, absence epilepsy and seizures. Fletcher, et al. (1996) Absence epilepsy in tottering mutant mice is associated with calcium channel defects. Cell

87:607-617; Burgess, et al. (1997) Mutation of the Ca2+ channel β subunit gene Cchb4 is associated with ataxia and seizures in the lethargic (lh) mouse. Cell 88:385-392; Ophoff, et al. (1996) Familial hemiplegic migraine and episodic ataxia type-2 are caused by mutations in the Ca2+ channel gene CACNL1A4. cell 87:543-552; Zhuchenko, O. et al. (1997) Autosomal dominat cerebellar ataxia (SCA6) associated with the small polyglutamine expansions in the α1A-voltage-dependent calcium channel. Nature Genetics 15:62-69.

The clinical treatment of some disorders has been aided by the development of therapeutic calcium channel antagonists. Janis, et al. (1991) In Calcium Channels: Their Properties, Functions, Regulation and Clinical Relevance. CRC Press, London.

Native calcium channels have been classified by their electrophysiological and pharmacological properties as T, L, N, P and Q types (for reviews see McCleskey, et al. (1991) Functional properties of voltage-dependent calcium channels. Curr. Topics Membr. 39: 295-326, and Dunlap, et al. (1995) Exocytotic Ca^{2+} channels in mammalian central neurons. Trends Neurosci. 18:89-98.). T-type (or low voltage-activated) channels describe a broad class of molecules that transiently activate at negative potentials and are highly sensitive to changes in resting potential. The L, N, P and Q-type channels activate at more positive potentials and display diverse kinetics and voltage-dependent properties. There is some overlap in biophysical properties of the high voltage-activated channels, consequently pharmacological profiles are useful to further distinguish them. L-type channels are sensitive to dihydropyridine (DHP) agonists and antagonists, N-type channels are blocked by the *Conus geographus* peptide toxin, ω-conotoxin GVIA, and P-type channels are blocked by the peptide ω-agatoxin IVA from the venom of the funnel web spider, *Agelenopsis aperta*. A fourth type of high voltage-activated Ca channel (Q-type) has been described, although whether the Q- and P-type channels are distinct molecular entities is controversial (Sather et al. (1993) Distinctive biophysical and pharmacological properties of class A (B1) calcium channel α1 subunits. Neuron 11: 291-303; Stea, et al. (1994) Localization and functional properties of a rat brain α1A calcium channel reflect similarities to neuronal Q- and P-type channels. Proc Natl Acad Sci (USA) 91: 10576-10580.). Several types of calcium conductances do not fall

neatly into any of the above categories and there is variability of properties even within a category suggesting that additional calcium channels subtypes remain to be classified.

Biochemical analyses show that neuronal calcium channels are heterooligomeric complexes consisting of three distinct subunits (α_1, $\alpha_2\delta$ and β)(reveiwed by De Waard, et al. (1997) In Ion Channels. Volume 4, edited by Narahashi, T. Plenum Press, New York). The α_1 subunit is the major pore-forming subunit and contains the voltage sensor and binding sites for calcium channel antagonists. The mainly extracellular α_2 is disulphide-linked to the transmembrane δ subunit and both are derived from the same gene and are proteolytically cleaved *in vivo*. The β subunit is a non-glycosylated, hydrophilic protein with a high affinity of binding to a cytoplasmic region of the α_1 subunit. A fourth subunit, γ, is unique to L-type Ca channels expressed in skeletal muscle T-tubules. The isolation and characterization of γ-subunit-encoding cDNAs is described in US Patent No. 5,386,025 which is incorporated herein by reference.

Molecular cloning has revealed the cDNA and corresponding amino acid sequences of six different types of α_1 subunits (α_{1A}, α_{1B}, α_{1C}, α_{1D}, α_{1E} and α_{1S}) and four types of β subunits (β_1, β_2, β_3 and β_4)(reviewed in Stea, A., Soong, T.W. and Snutch, T.P. (1994) Voltage-gated calcium channels. PCT Patent Publication WO 95/04144, which is incorporated herein by reference, discloses the sequence and expression of α_{1E} calcium channel subunits. In Handbook of Receptors and Channels. Edited by R.A. North, CRC Press.).

The different classes of $\alpha 1$ and β subunits have been identified in different animals including, rat, rabbit and human and share a significant degree of amino acid conservation across species (for examples see: Castellano, et al. (1993) Cloning and expression of a third calcium channel β subunit. J. Biol. Chem. 268: 3450-3455; Castellano, et al. (1993) Cloning and expression of a neuronal calcium channel β subunit. J. Biol. Chem. 268: 12359-12366; Dubel, et al. (1992). Molecular cloning of the α_1 subunit of an ω-conotoxin-sensitive calcium channel. Proc. Natl. Acad. Sci. (USA) 89: 5058-5062; Fujita, et al.. (1993) Primary structure and functional expression of the ω-conotoxin-sensitive N-type calcium channel from rabbit brain. Neuron 10: 585-598; Mikami, et al.. (1989). Primary structure and functional

expression of the cardiac dihydropyridine-sensitive calcium channel. Nature 340: 230-233; Mori. et al. (1991) Primary structure and functional expression from complementary DNA of a brain calcium channel. Nature 350: 398-402; Perez-Reyes. et al. (1992). Cloning and expression of a cardiac/brain ß subunit of the L-type calcium channel. J. Biol. Chem. 267: 1792-1797; Pragnell. et al. (1991). Cloning and tissue-specific expression of the brain calcium channel β-subunit. FEBS Lett. 291: 253-258; Snutch. et al. (1991) Distinct calcium channels are generated by alternative splicing and are differentially expressed in the mammalian CNS. Neuron 7: 45-57; Soong, et al. (1993) Structure and functional expression of a member of the low voltage-activated calcium channel family. Science 260: 1133-1136; Tomlinson, et al. (1993) Functional properties of a neuronal class C L-type channel. Neuropharmacology 32: 1117-1126; Williams, et al. (1992) Structure and functional expression of α1, α2, and ß subunits of a novel human neuronal calcium channel subtype. Neuron 8: 71-84; Williams. et al. (1992) Structure and functional expression of an ω-conotoxin-sensitive human N-type calcium channel. Science 257: 389-395.

In some expression systems the α_1 subunits alone can form functional calcium channels although their electrophysiological and pharmacological properties can be differentially modulated by coexpression with any of the four β subunits. Until recently, the reported modulatory affects of β subunit coexpression were to mainly alter kinetic and voltage-dependent properties. More recently it has been shown that β subunits also play crucial roles in modulating channel activity by protein kinase A, protein kinase C and direct G-protein interaction. (Bourinet, et al. (1994) Voltage-dependent facilitation of a neuronal α1C L-type calcium channel. EMBO J. 13: 5032-5039; Stea, et al. (1995) Determinants of PKC-dependent modulation of a family of neuronal calcium channels. Neuron 15:929-940; Bourinet, et al. (1996) Determinants of the G-protein-dependent opioid modulation of neuronal calcium channels. Proc. Natl. Acad. Sci. (USA) 93: 1486-1491.)

The electrophysiological and pharmacological properties of the calcium channels cloned to date can be summarized as shown in Table 1. While the cloned α_1 subunits identified to date correspond to several of the calcium channels found in cells, they do not account for all types of calcium conductances described in native cells. For example,

they do not account for the various properties described for the heterogenous family described as T-type calcium channels. Furthermore, they do not account for novel calcium channels described in cerebellar granule cells or other types of cells. (Forti, et al (1993) Functional diversity of L-type calcium channels in rat cerebellar neurons. Neuron 10: 437-450; Tottene,et al. (1996). Functional diversity of P-type and R-type calcium channels in rat cerebellar neurons. J. Neurosci. 16: 6353-6363).

Because of the importance of calcium channels in cellular metabolism and human disease, it would be desirable to identify the remaining classes of α_1 subunits, and to develop expression systems for these subunits which would permit the study and characterization of these calcium channels, including the study of pharmacological modulators of calcium channel function. Thus, it is an object of the present invention to provide heretofor undisclosed calcium channels having novel α_1 subunits, including cell lines expressing these new calcium channels. It is a further object of the present invention to provide a method for testing these novel calcium channels using such cell lines.

SUMMARY OF THE INVENTION

The present invention provides partial sequences for a novel mammalian (human and rat sequences identified) calcium channel subunit which we have labeled as the α_{1I} subunit, and an additional novel human calcium channel which we have labeled as the α_{1H} subunit. This knowledge of the sequence of these two calcium channels permits the localization and recovery of the complete sequence from human cells, and the development of cell lines which express the novel calcium channels of the invention. These cells may be used for identifying compounds capable of acting as agonists or antagonists to the calcium channels.

BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1 shows aligned amino acid sequences for the *C. elegans* C54D2.5 α_1 calcium channel subunit and initially identified portions of the calcium channel subunits of the invention.

TABLE 1

	ω-conotoxin GVIA	1,4-dihydropyridines	cadmium	ω-agatoxin IVA	ω-conotoxin MVIIC	native Ca^{2+} channel type
α_{1A}	-	-	✓	✓	✓	P/Q-type
α_{1B}	✓	-	✓	-	✓	N-type
α_{1C}	-	✓	✓	-	-	L-type
α_{1D}	-	✓	✓	-	-	L-type
α_{1E}	-	-	✓	-	-	novel
α_{1S}	-	✓	✓	-	-	L-type

DESCRIPTION OF THE INVENTION

The present invention includes the following aspects for which protection is sought:

(a) novel human calcium channel subunits and DNA fragments encoding such subunits. It will be appreciated that polymorphic variations may be made or may exist in the DNA of some individuals leading to minor deviations in the DNA or amino acids sequences from those shown which do not lead to any substantial alteration in the function of the calcium channel. Such variations. including variations which lead to substitutions of amino acids having similar properties are considered to be within the scope of the present invention.

(b) polynucleotide sequences useful as probes in screening human cDNA libraries for genes encoding these novel calcium channel subunits. These probes can also be used in histological assay to determine the tissue distribution of the novel calcium channel subunits.

(c) eukaryotic cell lines expressing the novel calcium channel subunits. These cell lines can be used to evaluate compounds as pharmacological modifiers of the function of the novel calcium channel subunits.

(d) a method for evaluating compounds as pharmacological modifiers of the function of the novel calcium channel subunits using the cell lines expressing those subunits alone or in combination with other calcium channel subunits.

Further, since defects in the novel calcium channel subunits may be associated with a human genetic disease including, but not limited to; epilepsy, migraine, ataxia, schizophrenia, hypertension, arrhythmia, angina, depression, small lung carcinoma, Lambert-Eaton syndrome, characterization of such associations and ultimately diagnosis of associated diseases can be carried out with probes which bind to the wild-type or defective forms of the novel calcium channels.

In accordance with the present invention, we have identified human DNA sequences which code for novel calcium channel α_1 subunits. These subunits are believed to represent two new types of α_1 subunits of human voltage-dependent calcium channels which have been designated as type α_{1I} and type α_{1H}.

The novel α_1 subunits of the invention were identified by screening the *C. elegans* genomic DNA sequence data base for sequences homologous to previously identified mammalian calcium channel α_1 subunits. Specifically, the following twelve mammalian α_1 subunit sequences were used to screen the *C. elegans* genomic data bank:

rat brain α_{1A} : GTCAAAACTC AGGCCTTCTA CTGG	SEQ ID. No. 1
rat brain α_{1A} : AACGTGTTCT TGGCTATCGC GGTG	SEQ ID. No. 2
rat brain α_{1B} : GTGAAAGCAC AGAGCTTCTA CTGG	SEQ ID. No. 3
rat brain α_{1B} : AACGTTTTCT TGGCCATTGC TGTG	SEQ ID. No. 4
rat brain α_{1C} : GTTAAATCCA ACGTCTTCTA CTGG	SEQ ID. No. 5
rat brain α_{1C} : AATGTGTTCT TGGCCATTGC GGTG	SEQ ID. No. 6
rat brain α_{1D} : GTGAAGTCTG TCACGTTTTA CTGG	SEQ ID. No. 7
rat brain α_{1D} : AAGCTCTTCT TGGCCATTGC TGTA	SEQ ID. No. 8
rat brain α_{1E} : GTCAAGTCGC AAGTGTTCTA CTGG	SEQ ID. No. 9
rat brain α_{1E} : AATGTATTCT TGGCTATCGC TGTG	SEQ ID. No. 10
rat brain consensus #1 : ATCTAYGCYR TSATYGGSAT G	SEQ ID. No. 11

rat brain consensus #2 : ATGGACAAYT TYGASTAYTC SEQ ID. No. 12

This search identified four distinct *C. elegans* cosmids that contain open reading frames (coding regions) that exhibit homology to mammalian calcium channel α_1 subunits:

cosmid and reading frame T02C5.5

cosmid and reading frame C48A7.1

cosmid and reading frame C54D2.5

cosmid and reading frame C27F2.3

Examination of the four *C. elegans* cosmid sequences by phylogeny analysis shows that two of these, T02C5.5 and C48A7.1, correspond closely with previously identified mammalian α_1 subunits. T02C5.5 appears to be an ancestral member related to the mammalian α_{1A}, α_{1B} and α_{1E} subunits. C48A7.1 appears to be an ancestral member related to the mammalian L-type channels encoded by α_{1C}, α_{1D} and α_{1S}. In contrast, the *C. elegans* cosmids C54D2.5 and C27F2.3 identify novel types of calcium channel α_1 subunits distinct from the other mammalian subtypes.

Mammalian counterparts of the *C. elegans* calcium channel α_1 subunit encoded by C54D2.5 were identified by screening of the GenBank expressed sequence tag (EST) data bank. This analysis identified a total of 13 mammalian sequences that exhibit some degree of DNA sequence and amino acid identity to C54D2.5, of which 8 are human sequences. (Table 2) Three of these sequences appear unlikely to encode novel calcium channel subunits because they either exhibit a significant degree of homology to previously identified mammalian α_1 subunits (clones H06096 and H14053) or exhibit homology in a region not considered to be diagnostic of calcium channel α_1 subunits specifically as opposed to other types of ion channel molecules in general (clone D20469). The five remaining sequences (H55225, H55617, H55223, H55544, and F07776), however, are believed to encode two previously unidentified calcium channel α_1 subunits because the degree of amino acid identity closely matches that of known calcium channel subunits in conserved regions but is sufficiently different to indicate that they do not encode previously identified mammalian

calcium channel α_1 subunits. α_{1A}, α_{1B}, α_{1C}, α_{1D}, α_{1E}, or α_{1S}. The expected amino acid sequence closely matches but is not identical to amino acid sequences in these known calcium channel subunits. The aligned amino acids sequences are shown in Fig 1.

Table 2

Query = C54D2.5 CE02562 CALCIUM CHANNEL ALPHA-1 SUBUNIT LG:6

Database: Non-redundant Database of GenBank EST Division
824,500 sequences; 302,742,428 total letter

Sequences producing High-scoring Segment Pairs:	Frame	Score	P(N)
gb|AA183990|AA183990 ms53e02.r1 Life Tech mouse embry...	+1	108	1.8e-24
gb|H55225|H55225 CHR220164 Homo sapiens genomic c...	+1	136	2.5e-10
dbj|D68412|CELK131B1F C.elegans cDNA clone yk131b1 : 5...	+3	117	1.7e-06
gb|R75128|R75128 MDB1075 Mouse brain, Stratagene ...	+3	113	7.2e-06
gb|H55617|H55617 CHR220556 Homo sapiens genomic c...	+2	102	2.8e-05
emb|F07776|HSC2HD061 H. sapiens partial cDNA sequence...	+3	100	0.00057
gb|W76774|W76774 me84e08.r1 Soares mouse embryo N...	+2	98	0.0012
gb|H06096|H06096 yl77e01.r1 Homo sapiens cDNA clo...	+3	98	0.0015
gb|H14053|H14053 ym65d10.r1 Homo sapiens cDNA clo...	+2	91	0.0036
gb|H55223|H55223 CHR220162 Homo sapiens genomic c...	+2	87	0.0039
dbj|D35703|CELK024D9F C.elegans cDNA clone yk24d9 : 5'...	+3	74	0.046
dbj|D20469|HUMGS01443 Human HL60 3'directed MboI cDNA,...	-2	66	0.91
gb|H55544|H55544 CHR220483 Homo sapiens genomic c...	+1	65	0.98

Four of the five sequences (H55225, H55617, H55223, and H55544) are found on human chromosome 22. and are now believed to all be part of the same gene encoding the

novel human calcium channel subunit α_{1I}. The fifth sequence, F07776 is apparently distinct and associated with a further novel human calcium channel subunit designated α_{1H}.

The sequences of the five selected sequences and the references from which they are taken are given as follows:

H55225 SOURCE human clone=C22_207 primer=T3 library=Chromosome 22 exon

Trofatter, et al., *Genome Res.* 5 (3): 214-224 (1995)

SEQ ID No. 13

1 GTGATCACTC TGGAAGGCTG GGTGGAGATC ATGTACTACG TGATGGATGC TCACTCCTTC

61 TACAACTTCA TCTACTTCAT CCTGCTTATC ATACCCCTCT TGCCTTGCAC CCCATATGGT

121 CTTCCCAGAG TGAGCTCATC CACCTCGTCA TGCCTGACTC GACGTTCA

H55617 SOURCE human clone=C22_757 primer=T3 library=Chromosome 22 exon

Trofatter, et al., *Genome Res.* 5 (3): 214-224 (1995)

SEQ ID No. 14

1 GATGGTCGAG TACTCCCTGG ACCTTCAGAA CATCAACCTG TCAGCCATCC GCACCGTGCG

61 CGTCCTGAGG CCCCTCAAAG CCATCAACCG CGTGCCCA

H55223 SOURCE human clone=C22_204 primer=T3 library=Chromosome 22 exon

Trofatter, et al, *Genome Res.* 5 (3): 214-224 (1995)

SEQ ID No. 15

1 CATGCTGGTG ATCCTGCTGA ACTGCGTGAC ACTTGGCATG TACCAGCCGT GCGACGACAT

61 GGACTGCCTG TCCGACCGCT GCAAGATCCT GCAG

H55544 SOURCE human clone=C22_651 primer=T3 library=Chromosome 22 exon

Trofatter. et al. *Genome Res.* 5 (3): 214-224 (1995)

SEQ ID No. 16

1 GTATCTCTGG TTACTTTAGT AGCCAACACT CTTGGCTACT CAGACCTTGG TCCCATTAAA

61 TCCCTGCGAA CCTTGAGAGC ACTAAGACCT CTAAGAGCTT TGTCTAGATT TGAAGGAATG

121 AGG

F07776 SOURCE human.

Submitted (19-JAN-1995) Genethon. B.P. 60, 91002 Evry Cedex France and Genetique Moleculaire et Biologie du developpement, CNRS UPR420 B.P. 8, 94801 Villejuif Cedex France E-mail: genexpress@genethon.fr

SEQ ID No. 17

1 TTCTCTCCAT TGTAGGAATG TTTCTGGCTG AACTGATAGA AAAGTATTTT GTGTGCCCTA

61 CCCTGTTNCG AGTGATCCGT CTTGCCAGGA TTGGCCGAAT CCTACGTCTG ATCAAAGGAG

121 CAAAGGGGAT CCGCACGCTG CTCTTTGCTT TGATGATGTC CCTTCCTGCG TTGTTTAACA

181 TCGGNCTCCT TCTTTTCCTG GTCATGTTCA TCTACGNCAT CTTTGGGATG TCCAATTTTG

241 CCTATGTTAA GAGGGAAGTT GGGATCGATG ACATGTTNAN CTTTGAGACC TTTGGCAACA

301 GCATGATCTG CCTGTTCCAA ATTACAACCT CTGCTGGCTG GGA

A search of the Sanger Genome Sequencing Center (Cambridge, U.K.) and the Washington University Genome Sequencing Center (St. Louis. MO) sequences in progress revealed a Bacterial Artificial Chromosome (BAC) sequence (bK206c7) that contained matches to the *C. elegans* cosmid open reading frame, C54D2.5, and to the four human chromosome 22 ESTs, H55225, H55617, H55223,H55544. The C. elegans C54D2.5 cosmid sequence and the human EST sequences were then used to compare the translation of the

bK206c7 BAC genomic sequence in all 6 reading frames. The analysis was performed using the graphical program Dotter (Eric Sohnhammer. NCBI). The analysis revealed a series of potential coding regions on one strand of the bK206c7 BAC sequence. These were subsequently translated in all 3 reading frames and the potential splice junctions identified. The translated sequence of this longer DNA fragment which is part of the human α_{11} subunit gene is given by Seq. ID No. 18.

Using the sequence information from the five EST's, a full length gene can be recovered using any of several techniques. Polynucleotide probes having a sequence which corresponds to or hybridizes with the EST sequences or a distinctive portion thereof (for example oligonucleotide probes having a length of 18 to 100 nucleotides) can be used to probe a human cDNA library for identification of the full length DNA encoding the α_{11} and α_{1H} subunits. The process of identifying cDNAs of interest using defined probes is well known in the art and is, for example, described in International Patent Publication No. WO95/04144, which is incorporated herein by reference. This process generally involves screening bacterial hosts (e.g. *E. coli*) harboring the library plasmids or infected with recombinant lambda phage with labeled probes, e.g. radiolabeled with ^{32}P, and selection of colonies or phage which bind the labeled probe. Each selected colony or phage is grown up, and the plasmids are recovered. Human cDNAs are recovered from the plasmids by restriction digestion, or can be amplified, for example by PCR. The recovered cDNA can be sequenced, and the position of the calcium channel subunit-encoding region further refined, although neither process is not necessary to the further use of the cDNA to produce cell lines expressing the novel calcium channel subunits.

Longer portions of DNA-encoding the novel calcium channel subunits of the invention can also be recovered by PCR cloning techniques using primers corresponding to or based upon the EST sequences. Using this technique to identify relevant sequences within a human brain total RNA preparation confirmed that the novel α_{11} calcium channel subunit is present in human brain. Subcloning of the 567 nt PCR product and subsequent sequencing thereof showed that this product corresponds to the derived sequence form the bK206c7 BAC genomic sequence. The nucleotide sequence is given as SEQ ID No. 19. The same

experiment was performed using a rat brain RNA preparation and resulted in recovery of a substantially identical PCR product. (SEQ ID. NO. 20). The protein encoded by the rat PCR product is 96% identical to the human PCR product.

These sequences. which presumably encode a partial subunit can be used as a basis for constructing full length human or rat α_{II} clones. Briefly, the subcloned α_{II} PCR product is radiolabeled by random hexamer priming according to standard methods (See, Sambrook , J., Fritsch, E.F. and Maniatis, T. (1989) Molecular Cloning, A Laboratory Manual. Cold Spring Harbor Press) and used to screen commercial human brain cDNA libraries (Stratagene. La Jolla, CA). The screening of cDNA libraries follows standard methods and includes such protocols as infecting bacteria with recombinant lambda phage, immobilizing lambda DNA to nitrocellulose filters and screening under medium hybridization stringency conditions with radiolabeled probe. cDNA clones homologous to the probe are identified by autoradiography. Positive clones are purified by sequential rounds of screening.

Following this protocol, most purified cDNA's are likely to be partial sequence clones due the nature of the cDNA library synthesis. Full length clones are constructed from cDNA's which overlap in DNA sequence. Restriction enzyme sites which overlap between cDNAs are used to ligate the individual cDNA's to generate a full-length cDNA. For subsequent heterologous expression, the full-length cDNA is subcloned directly into an appropriate vertebrate expression vector. such as pcDNA-3 (Invitrogen, San Diego, CA) in which expression of the cDNA is under the control of a promoter such as the CMV major intermediate early promoter/enhancer. Other suitable expression vectors include, for example, pMT2, pRC/CMV, pcDNA3.1 and pCEP4.

Once the full length cDNA is cloned into an expression vector, the vector is then transfected into a host cell for expression. Suitable host cells include *Xenopus* oocytes or mammalian cells such as human embryonic kidney cells as described in International Patent Publication No. WO 96/39512 which is incorporated herein by reference and Ltk cells as described in US Patent No. 5,386.025 which is incorporated herein by reference. Transfection into host cells may be accomplished by microinjection, lipofection, glycerol shock, electroporation calcium phosphate or particle-mediated gene transfer. The vector may also be

transfected into host cells to provide coexpression of the novel α_1 subunits with a β and/or an $\alpha_2\delta$ subunit.

The resulting cell lines expressing functional calcium channels including the novel α_1 subunits of the invention can be used test compounds for pharmacological activity with respect to these calcium channels. Thus, the cell lines are useful for screening compounds for pharmaceutical utility. Such screening can be carried out using several available methods for evaluation of the interaction, if any, between the test compound and the calcium channel. One such method involves the binding of radiolabeled agents that interact with the calcium channel and subsequent analysis of equilibrium binding measurements including but not limited to, on rates, off rates, K_d values and competitive binding by other molecules. Another such method involves the screening for the effects of compounds by electrophysiological assay whereby individual cells are impaled with a microelectrode and currents through the calcium channel are recorded before and after application of the compound of interest. Another method, high-throughput spectrophotometric assay, utilizes the loading the cell lines with a fluorescent dye sensitive to intracellular calcium concentration and subsequent examination of the effects of compounds on the ability of depolarization by potassium chloride or other means to alter intracellular calcium levels. Compounds to be tested as agonists or antagonists of the novel α_{1I} and α_{1H} calcium channel subunits are combined with cells that are stably or transiently transformed with a DNA sequence encoding the α_{1I} or α_{1H} calcium channel subunits of the invention and monitored using one of these techniques.

DNA fragments with sequences given by SEQ ID Nos. 13-19 may also be used for mapping the distribution of α_{1I} and α_{1H} calcium channel subunits within a tissue sample. This method follows normal histological procedures using a nucleic acid probe, and generally involves the steps of exposing the tissue to a reagent comprising a directly or indirectly detectable label coupled to a selected DNA fragment, and detecting reagent that has bound to the tissue. Suitable labels include fluorescent labels, enzyme labels, chromophores and radio-labels.

EXAMPLE 1

In order to isolate novel human calcium channel α_1 subunits using standard molecular cloning protocols, synthetic DNA probes are prepared, radiolabeled with ^{32}P and utilized to screen human cDNA libraries commercially available in lambda phage vectors (Stratagene, La Jolla, CA) based on the human DNA sequences for H55225, H55617, H55223, H55544 and F07776. DNA fragments with the sequence of sequence ID NOs 18 and 19 may also be used for this purpose. Positive phage are purified through several rounds of screening involving immobilizing the phage DNA on nitrocellulose filters, hybridizing with the radiolabeled probe, washing off of excess probe and then selection of clones by autoradiography. Clones identified by this approach are expected to be partial length clones due to the nature of cDNA library synthesis and several rounds of screening for each calcium channel type may be necessary to obtain full-length clones.

To characterize the clones, double stranded plasmid DNA is prepared from the identified clones and the sequences are determined using ^{35}S dATP, Sequenase and standard gel electrophoresis methods. Regions of similarity and regions of overlap are determined by comparison of each cDNA sequence.

Full-length clones are constructed by ligating overlapping cDNA fragments together at common restriction enzyme sites. The full-length clones are subsequently inserted into vectors suitable for expression in vertebrate cells (e.g. pMT2, pRC/CMV, pcDNA3.1, pCEP4, pREP7) by ligation into restriction sites in the vector polylinker region which is downstream of the promoter used to direct cDNA expression.

DNA encoding the novel calcium channels can be stably or transiently introduced into eukaryotic cells (e.g. human embryonic kidney, mouse L cells, chinese hamster ovary, etc) by any number of available standard methods. Stable transfection is achieved by growing the cells under conditions that promote growth of cells expressing a marker gene which is contained in the expression vector (e.g. dihydrofolate reductase, thymidine kinase, or the like). The heterologous DNA encoding the human calcium channel may be integrated into the genome or may be maintained as an episomal element.

Expression of the human calcium channel in transfected cells may monitored by any number of techniques. including Northern blot for RNA analysis. Southern blot for cDNA detection. electrophysiological assay for calcium channel function. the binding of radiolabeled agents thought to interact with the calcium channel. and fluorescent assay of dyes sensitive to intracellular calcium concentration.

EXAMPLE 2

Heterologous Expression of Human α_{1I} Calcium Channels in Cells

A. Transient Transfection in Mammalian Cells

Host cells, such as human embryonic kidney cells, HEK 293 (ATCC# CRL 1573) are grown in standard DMEM medium supplemented with 2 mM glutamine and 10% fetal bovine serum. HEK 293 cells are transfected by a standard calcium-phosphate-DNA co-precipitation method using the full-lenngth human α_{1I} calcium channel cDNA in a vertebrate expression vector (for example see Current protocols in Molecular Biology). The human α_{1I} calcium channel cDNA may be transfected alone or in combination with other cloned subunits for mammalian calcium channels, such as $\alpha 2\delta$ and β subunits, and also with clones for marker proteins such the jellyfish green fluorescent protein.

Electrophysiological Recording: After an incubation period of from 24 to 72 hrs the culture medium is removed and replaced with external recording solution (see below). Whole cell patch clamp experiments are performed using an Axopatch 200B amplifier (Axon Instruments, Burlingame, CA) linked to an IBM compatible personal computer equipped with pCLAMP software. Microelectrodes are filled with 3 M CsCl and have typical resistances from 0.5 to 2.5 M_. The external recording solution is 20 mM $BaCl_2$, 1 mM $MgCl_2$, 10 mM HEPES, 40 mM TEACl, 10 mM Glucose, 65 mM CsCl, (pH 7.2). The internal pipette solution is 105 mM CsCl, 25 mM TEACl, 1 mM $CaCl_2$, 11 mM EGTA, 10 mM HEPES (pH 7.2). Currents are typically elicited from a holding potential of -100 mV to various test potentials. Data are filtered at 1 kHz and recorded directly on the harddrive of a personal computer. Leak subtraction is carried out on-line using a standard P/5 protocol. Currents are

analyzed using pCLAMP versions 5.5 and 6.0. Macroscopic current-voltage relations are fitted with the equation $I = \{1/(1+\exp(-(V_m-V_h)/S)\} \times G - (V_m-E_{rev})$, where V_m is the test potential. V_h is the voltage at which half of the channels are activated. and S reflects the steepness of the activation curve and is an indication of the effective gating charge movement. Inactivation curves are normalized to 1 and fitted with $I = (1/1 + \exp((V_m-V_h)/S)$ with V_m being the holding potential. Single channel recordings are performed in the cell-attached mode with the following pipette solution (in mM): 100 $BaCl_2$, 10 HEPES, pH 7.4 and bath solution: 100 KCl, 10 EGTA, 2 $MgCl_2$, 10 HEPES, pH 7.4.

B. Transient Transfection in Xenopus Oocytes

Stage V and VI Xenopus oocytes are prepared as described by Dascal et al (1986), Expression and modulation of voltage-gated calcium channels after RNA injection into Xenopus oocytes. Science 231:1147-1150. After enzymatic dissociation with collagenase, oocytes nuclei are microinjected with the human α_{1I} calcium channel cDNA expression vector construct (approximately 10 ng DNA per nucleus) using a Drummond nanoject apparatus. The human α_{1I} calcium channel may be injected alone, or in combination with other mammalian calcium channel subunit cDNAs, such as the $\alpha 2$-δ and $\beta 1b$ subunits. After incubation from 48 to 96 hrs macroscopic currents are recorded using a standard two microelectrode voltage-clamp (Axoclamp 2A, Axon Instruments, Burlingame. CA) in a bathing medium containing (in mM): 40 $Ba(OH)_2$, 25 TEA-OH, 25 NaOH, 2 CsOH, 5 HEPES (pH titrated to 7.3 with methan-sulfonic acid). Pipettes of typical resistance ranging from 0.5 to 1.5 m_ are filled with 2.8M CsCl, 0.2M CsOH, 10mM HEPES, 10mM BAPTA free acid. Endogenous Ca (and Ba) -activated Cl currents are suppressed by systematically injecting 10-30 nl of a solution containing 100mM BAPTA-free acid, 10mM HEPES (pH titrated to 7.2 with CsOH) using a third pipette connected to a pneumatic injector. Leak currents and capacitive transients are subtracted using a standard P/5 procedure.

EXAMPLE 3

Construction of Stable Cell Lines Expressing Human α_{1I} Calcium Channels

Mammalian cell lines stably expressing human α_{1I} calcium channels are constructed by transfecting the α_{1I} calcium channel cDNA into mammalian cells such as HEK 293 and selecting for antibiotic resistance encoded for by an expression vector. Briefly, the full-length human α_{1I} calcium channel cDNA subcloned into a vertebrate expression vector with a selectable marker, such as the pcDNA3 (InvitroGen, San Diego, CA), is transfected into HEK 293 cells by calcium phosphate coprecipitation or lipofection or electroporation or other method according to well known procedures (Methods in Enzymology, Volume 185, Gene Expression Technology (1990) Edited by Goeddel, D.V.). The human α_{1I} calcium channel may be transfected alone, or in combination with other mammalian calcium channel subunit cDNAs, such as the $\alpha 2$-δ and $\beta 1b$ subunits, either in a similar expression vector or other type of vector using different selectable markers. After incubation for 2 days in nonselective conditions, the medium is supplemented with Geneticin (G418) at a concentration of between 600 to 800 ug/ml. After 3 to 4 weeks in this medium, cells which are resistant to G418 are visible and can be cloned as isolated colonies using standard cloning rings. After growing up each isolated colony to confluency to establish cell lines, the expression of human α_{1I} calcium channels can be determined at with standard gene expression methods such as Northern blotting, RNase protection and reverse-transcriptase PCR.

The functional detection of human α_{1I} calcium channels in stably transfected cells can be examined electrophysiologically, such as by whole patch clamp or single channel analysis (see above). Other means of detecting functional calcium channels include the use of radiolabeled ^{45}Ca uptake, fluorescence spectroscopy using calcium sensitive dyes such as FURA-2, and the binding or displacement of radiolabeled ligands that interact with the calcium channel.

SEQUENCE LISTING

(1) GENERAL INFORMATION:
(i) APPLICANT: Snutch, Terry P.
Baillie, David L.

(ii) TITLE OF INVENTION: Novel Human Calcium Channels and Related Probes, Cell Lines and Methods
(iii) NUMBER OF SEQUENCES: 20
(iv) CORRESPONDENCE ADDRESS:
(A) ADDRESSEE:
(B) STREET:
(C) CITY:
(D) STATE:
(E) COUNTRY:
(F) ZIP:
(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Diskette, 3.50 inch, 1.44 Kb storage
(B) COMPUTER: IBM Compatible
(C) OPERATING SYSTEM: MS DOS 6.0
(D) SOFTWARE: WordPerfect
(vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER:
(B) FILING DATE:
(C) CLASSIFICATION:
(viii) ATTORNEY/AGENT INFORMATION:
(A) NAME: Larson, Marina T.
(B) REGISTRATION NUMBER: 32038
(C) REFERENCE/DOCKET NUMBER: NMED.P-001-US
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(2) INFORMATION FOR SEQ ID NO:1:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii)MOLECULE TYPE: other nucleic acid
(iii) HYPOTHETICAL: no
(iv) ANTI-SENSE: no
(vi) ORIGINAL SOURCE:
(A) ORGANISM: rat
(ix) FEATURE: oligonucleotide probe for locating calcium channel genes
(xi)SEQUENCE DESCRIPTION: SEQ ID NO:1:
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(ii)MOLECULE TYPE: other nucleic acid
(iii) HYPOTHETICAL: no
(iv) ANTI-SENSE: no
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(A) ORGANISM: rat
(ix) FEATURE: oligonucleotide probe for locating calcium channel genes
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(D) TOPOLOGY: linear
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(iv) ANTI-SENSE: no
(vi) ORIGINAL SOURCE:
(A) ORGANISM: rat
(ix) FEATURE: oligonucleotide probe for locating calcium channel genes
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(D) TOPOLOGY: linear
(ii)MOLECULE TYPE: other nucleic acid
(iii) HYPOTHETICAL: no
(iv) ANTI-SENSE: no
(vi) ORIGINAL SOURCE:
(A) ORGANISM: rat
(ix) FEATURE: oligonucleotide probe for locating calcium channel genes
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(D) TOPOLOGY: linear
(ii)MOLECULE TYPE: other nucleic acid
(iii) HYPOTHETICAL: no
(iv) ANTI-SENSE: no
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(A) ORGANISM: rat
(ix) FEATURE: oligonucleotide probe for locating calcium channel genes
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(D) TOPOLOGY: linear
(ii)MOLECULE TYPE: other nucleic acid
(iii) HYPOTHETICAL: no
(iv) ANTI-SENSE: no
(vi) ORIGINAL SOURCE:
(A) ORGANISM: rat
(ix) FEATURE: oligonucleotide probe for locating calcium channel genes
(xi)SEQUENCE DESCRIPTION: SEQ ID NO: 6:
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(D) TOPOLOGY: linear
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(iii) HYPOTHETICAL: no
(iv) ANTI-SENSE: no
(vi) ORIGINAL SOURCE:
(A) ORGANISM: rat

(ix) FEATURE: oligonucleotide probe for locating calcium channel genes
(xi)SEQUENCE DESCRIPTION: SEQ ID NO: 7:
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(D) TOPOLOGY: linear
(ii)MOLECULE TYPE: other nucleic acid
(iii) HYPOTHETICAL: no
(iv) ANTI-SENSE: no
(vi) ORIGINAL SOURCE:
(A) ORGANISM: rat
(ix) FEATURE: oligonucleotide probe for locating calcium channel genes
(xi)SEQUENCE DESCRIPTION: SEQ ID NO: 8:
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(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii)MOLECULE TYPE: other nucleic acid
(iii) HYPOTHETICAL: no
(iv) ANTI-SENSE: no
(vi) ORIGINAL SOURCE:
(A) ORGANISM: rat
(ix) FEATURE: oligonucleotide probe for locating calcium channel genes
(xi)SEQUENCE DESCRIPTION: SEQ ID NO: 9:
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(2) INFORMATION FOR SEQ ID NO: 10:
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(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii)MOLECULE TYPE: other nucleic acid
(iii) HYPOTHETICAL: no
(iv) ANTI-SENSE: no

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(A) ORGANISM: rat
(ix) FEATURE: oligonucleotide probe for locating calcium channel genes
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(A) ORGANISM: rat
(ix) FEATURE: oligonucleotide probe for locating calcium channel genes
(xi)SEQUENCE DESCRIPTION: SEQ ID NO: 11:
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(B) TYPE: nucleic acid
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(D) TOPOLOGY: linear
(ii)MOLECULE TYPE: other nucleic acid
(iii) HYPOTHETICAL: no
(iv) ANTI-SENSE: no
(vi) ORIGINAL SOURCE:
(A) ORGANISM: rat
(ix) FEATURE: oligonucleotide probe for locating calcium channel genes
(xi)SEQUENCE DESCRIPTION: SEQ ID NO: 12:
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(2) INFORMATION FOR SEQ ID NO: 13:
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(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii)MOLECULE TYPE: other nucleic acid

(iii) HYPOTHETICAL: no
(iv) ANTI-SENSE: no
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(A) ORGANISM: human
(ix) FEATURE: expressed sequence tag H55225
(xi)SEQUENCE DESCRIPTION: SEQ ID NO: 13:

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(D) TOPOLOGY: linear
(ii)MOLECULE TYPE: other nucleic acid
(iii) HYPOTHETICAL: no
(iv) ANTI-SENSE: no
(vi) ORIGINAL SOURCE:
(A) ORGANISM: human
(ix) FEATURE: expressed sequence tag H55617
(xi)SEQUENCE DESCRIPTION: SEQ ID NO: 14:

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(iv) ANTI-SENSE: no
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(A) ORGANISM: human
(ix) FEATURE: expressed sequence tag H55223
(xi)SEQUENCE DESCRIPTION: SEQ ID NO: 15:

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(D) TOPOLOGY: linear
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(vi) ORIGINAL SOURCE:
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(ix) FEATURE: expressed sequence tag H55544
(xi)SEQUENCE DESCRIPTION: SEQ ID NO: 16:

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(iv) ANTI-SENSE: no
(vi) ORIGINAL SOURCE:
(A) ORGANISM: human
(ix) FEATURE: expressed sequence tag F07776
(xi)SEQUENCE DESCRIPTION: SEQ ID NO: 17:

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CAAAGGGGAT CCGCACGCTG CTCTTTGCTT TGATGATGTC CCTTCCTGCG TTGTTTAACA   180
TCGGNCTCCT TCTTTTCCTG GTCATGTTCA TCTACGNCAT CTTTGGGATG TCCAATTTTG   240
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(2) INFORMATION FOR SEQ ID NO: 18:
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(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii)MOLECULE TYPE: cDNA
(iii) HYPOTHETICAL: no
(iv) ANTI-SENSE: no
(vi) ORIGINAL SOURCE:
(A) ORGANISM: human
(ix) FEATURE: human alpha-I partial sequence from BAC bK206c7
(xi)SEQUENCE DESCRIPTION: SEQ ID NO: 18:

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Val Ala Asn Met Asp Asn Phe Phe Ala Pro Val Phe Thr Met Gly Lys Tyr Tyr

ACG CAA GGC GAC AAG GTG CTG ATG CCG CTG GCG ATT CAG GCT CTG AAA CAG CTG   162
Thr Gln Gly Asp Lys Val Leu Met Pro Leu Ala Ile Gln Ala Leu Lys Gln Leu

ATG TTC AAA TTG GTG GCC ACT GTT GCT CGA ACA CAT GCT ACA CCG TCA CAC ATC   206
Met Phe Lys Leu Val Ala Thr Val Ala Arg Thr His Ala Thr Pro Ser His Ile

ACG GGT GGT CCT GGA ACA GGG ATG CAC ACG GGC ACC TTC CAG GAA GGA GCT GAG   270
Thr Gly Gly Pro Gly Thr Gly Met His Thr Gly Thr Phe Gln Glu Gly Ala Glu

CCT GGT TCA TCT CAG CAC CCT GAG GCA CAG GCC ACG TAT ACA GCA GGG TGC ACC   324
Pro Gly Ser Ser Gln His Pro Glu Ala Gln Ala Thr Tyr Thr Ala Gly Cys Thr

CCA GCC CCC ACG GGC GAT CCC ACC TGC TGC TTT GTC CTT GAC TTG GTG TGC ACG   378
Pro Ala Pro Thr Gly Asp Pro Thr Cys Cys Phe Val Leu Asp Leu Val Cys Thr

TGG TTT GAA TGT GTC AGC ATG CTG GTG ATC CTG CTG AAC TGC GTG ACA CTT GGC   432
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ATG GTG GCC CTG GGG ATT TTT GGC AAG AAG TGC TAC CTC GGG GAC ACA TGG AAC   594
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ACC GTG CGC GTC CTG AGG CCC CTC AAA GCC ATC AAC CGC GTG CCC AGT ATG CGG   702
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ATC CTG GTG AAC CTG CTC CTG GAC ACA CTG CCC ATG CTG GGG AAT GTC CTG CTG   756
Ile Leu Val Asn Leu Leu Leu Asp Thr Leu Pro Met Leu Gly Asn Val Leu Leu

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Val Ala Leu Pro Pro Tyr Tyr Gln Pro Glu Glu Asp Asp Glu Met Pro Phe Ile

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AAG GAG CAG GGC CGT GAG TGC TGC CTG TCC AAG GAC GAC GTC TAC GAC TTT GGG  1026
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GCG GGG CGC CAG GAC CTC AAT GCC AGC GGC CTC TGT GTC AAC TGG AAC CGT TAC  1080
Ala Gly Arg Gln Asp Leu Asn Ala Ser Gly Leu Cys Val Asn Trp Asn Arg Tyr

TAC AAT GTG TGC CGC ACG GGC AGC GCC AAC CCC CAC AAG GGT GCC ATC AAC TTT  1134
Tyr Asn Val Cys Arg Thr Gly Ser Ala Asn Pro His Lys Gly Ala Ile Asn Phe

GAC AAC ATC GGT TAT GCT TGG ATT GTC ATC TTC CAG GTG ATC ACT CTG GAA GGC  1188
Asp Asn Ile Gly Tyr Ala Trp Ile Val Ile Phe Gln Val Ile Thr Leu Glu Gly

TGG GTG GAG ATC ATG TAC TAC GTG ATG GAT GCT CAC TCC TTC TAC AAC TTC ATC  1242
Trp Val Glu Ile Met Tyr Tyr Val Met Asp Ala His Ser Phe Tyr Asn Phe Ile

TAC TTC ATC CTG CTT ATC ATA AGT GAG CTC ATC CAC CTC GTC ATG CCT GAC TGC  1296
Tyr Phe Ile Leu Leu Ile Ile Ser Glu Leu Ile His Leu Val Met Pro Asp Cys

AGC TTC AGC ACA GCA CAG TCC CCA AAA TGT CAA GGT GAT TCA CTC CCA GGA GTC  1350
Ser Phe Ser Thr Ala Gln Ser Pro Lys Cys Gln Gly Asp Ser Leu Pro Gly Val
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GCT GCT GAA TCC CTG CTG CTG CGA GAC TCT AGC TCC TCA GTC ATC ACT GAT GAG 1404
Ala Ala Glu Ser Leu Leu Leu Arg Asp Ser Ser Ser Ser Val Ile Thr Asp Glu

GCT GCA GCC ATG GAG AAC CTC CTG GCG GGC ACC TCC AAG GGG GAT GAA AGC TAT 1458
Ala Ala Ala Met Glu Asn Leu Leu Ala Gly Thr Ser Lys Gly Asp Glu Ser Tyr

CTG CTC AGG CTG GCC GGC AGC CAA GTT CAC TCC CAG GCT CAG CAA ATG CTG GGG 1512
Leu Leu Arg Leu Ala Gly Ser Gln Val His Ser Gln Ala Gln Gln Met Leu Gly

AGG GGG CTG GGC CCT GAA AGC CTG GAA ACT GGA GAG GAG CCC CAC TCG TGG AGC 1566
Arg Gly Leu Gly Pro Glu Ser Leu Glu Thr Gly Glu Glu Pro His Ser Trp Ser

CCT CGG GCC ACA AGG AGA TGG GAT CCC CAA TGC CAA CCA GGG CAG CCT CTC CCC 1620
Pro Arg Ala Thr Arg Arg Trp Asp Pro Gln Cys Gln Pro Gly Gln Pro Leu Pro

CTT CAT TTC ATG CAA GCA CAG GTG GGC TCC TTC TTC ATG ATC AAC CTG TGC CTC 1674
Leu His Phe Met Gln Ala Gln Val Gly Ser Phe Phe Met Ile Asn Leu Cys Leu

GTT GTC ATA GCG ACC CAG TTC TCG GAG ACC AAG CAA CGG GAG CAC CGG CTG ATG 1728
Val Val Ile Ala Thr Gln Phe Ser Glu Thr Lys Gln Arg Glu His Arg Leu Met

CTG GAG CAG CGG CAG CGC TAC CTG TCC TCC AGC ACG GTG GCC AGC TAC GCC GAG 1782
Leu Glu Gln Arg Gln Arg Tyr Leu Ser Ser Ser Thr Val Ala Ser Tyr Ala Glu

CCT GGC GAC TGC TAC GAG GAG ATC TTC CAG TAT GTC TGC CAC ATC CTG CGC AAG 1836
Pro Gly Asp Cys Tyr Glu Glu Ile Phe Gln Tyr Val Cys His Ile Leu Arg Lys

GCC AAG CGC CGC GCC CTG GGC CTC TAC CAG GCC CTG CAG AGC CGG CGC CAG GCC 1890
Ala Lys Arg Arg Ala Leu Gly Leu Tyr Gln Ala Leu Gln Ser Arg Arg Gln Ala

CTG GGC CCG GAG GCC CCG GCC CCC GCC AAA CCT GGG CCC CAC GCC AAG GAG CCC 1944
Leu Gly Pro Glu Ala Pro Ala Pro Ala Lys Pro Gly Pro His Ala Lys Glu Pro

CGG CAC TAC CCT CTC ACA GTC TGG GAA TCG ATT CTT GGG AGG CAA GCA GAA GAA 1998
Arg His Tyr Pro Leu Thr Val Trp Glu Ser Ile Leu Gly Arg Gln Ala Glu Glu

TGC ACG CTC AGA GCT GCC GCC CAC CCG TCC TCG GGT GCC AGC CAT CCA GGC GTG 2049
Cys Thr Leu Arg Ala Ala Ala His Pro Ser Ser Gly Ala Ser His Pro Gly Val

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GGC TCG GAG GAG GCC CCA GAG CTG TGC CCG CAA CAT AGC CCC CTG GAT GCG ACG  2106
Gly Ser Glu Glu Ala Pro Glu Leu Cys Pro Gln His Ser Pro Leu Asp Ala Thr

CCC CAC ACC CTG GTG CAG CCC ATC CCC GCC ACG CTG GCT TCC GAT CCC GCC AGC  2160
Pro His Thr Leu Val Gln Pro Ile Pro Ala Thr Leu Ala Ser Asp Pro Ala Ser

TGC CCT TGC TGC CAG CAT GAG GAC GGC CGG CGG CCC TCG GGC CTG GGC AGC ACC  2214
Cys Pro Cys Cys Gln His Glu Asp Gly Arg Arg Pro Ser Gly Leu Gly Ser Thr

GAC TCG GGC CAG GAG GGC TCG GGC TCC GGG AGC TCC GCT GGT GGC GAG GAC GAG  2268
Asp Ser Gly Gln Glu Gly Ser Gly Ser Gly Ser Ser Ala Gly Gly Glu Asp Glu

GCG GAT GGG GAC GGG GCC CGG AGC AGC GAG GAC GGA GCC TCC TCA GAA CTG GGG  2322
Ala Asp Gly Asp Gly Ala Arg Ser Ser Glu Asp Gly Ala Ser Ser Glu Leu Gly

AAG GAG GAG GAG GAG GAG GAG CAG GCG GAT GGG GCG GTC TGG CTG TGC GGG GAT  2376
Lys Glu Glu Glu Glu Glu Glu Gln Ala Asp Gly Ala Val Trp Leu Cys Gly Asp

GTG TGG CGG GAG ACG CGA GCC AAG CTG CGC GGC ATC GTG GAC AGC AAG TAC TTC  2430
Val Trp Arg Glu Thr Arg Ala Lys Leu Arg Gly Ile Val Asp Ser Lys Tyr Phe

AAC CGG GGC ATC ATG ATG GCC ATC CTG GTC AAC ACC GTC AGC ATG GGC ATC GAG  2484
Asn Arg Gly Ile Met Met Ala Ile Leu Val Asn Thr Val Ser Met Gly Ile Glu

CAC CAC GAG CAG GCC AGT GCA GCG CAG CCG GGC CGG GCC TGC GGG AGA GGA CAA  2538
His His Glu Gln Ala Ser Ala Ala Gln Pro Gly Arg Ala Cys Gly Arg Gly Gln

AAT CCA GAC CTT TGC ATG ACC CTC AAG GCC CCT TGT CTC TGT CAC AAC GTC CCT  2592
Asn Pro Asp Leu Cys Met Thr Leu Lys Ala Pro Cys Leu Cys His Asn Val Pro


TCA CCA GGC CAG GGT GTC CTG TCC CAT CCA GTG ACT CCA CCC CAT ACA GCC CCA  2646
Ser Pro Gly Gln Gly Val Leu Ser His Pro Val Thr Pro Pro His Thr Ala Pro

TGG CGC ATG GAG ACA GGA AAG CAG GGA CAC GGA TGT GAA GAA GGA CCA GGA CAA  2700
Trp Arg Met Glu Thr Gly Lys Gln Gly His Gly Cys Glu Glu Gly Pro Gly Gln

CGA AGC AGT GAC ATG TTT GCC CTG GAG ATG ATC CTG AAG CTG GCT GCA TTT GGG  2754
Arg Ser Ser Asp Met Phe Ala Leu Glu Met Ile Leu Lys Leu Ala Ala Phe Gly
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CTC TTC GAC TAC CTG CGT AAC CCC TAC AAC ATC TTC GAC AGC ATC ATT GTC ATC 2808
Leu Phe Asp Tyr Leu Arg Asn Pro Tyr Asn Ile Phe Asp Ser Ile Ile Val Ile

ATC AGC ATC TGG GAG ATC GTG GGG CAG GCG GAC GGT GGG CTG TCG GTG CTG CGG 2862
Ile Ser Ile Trp Glu Ile Val Gly Gln Ala Asp Gly Gly Leu Ser Val Leu Arg

ACC TTC CGG CTG CTG CGC GTG CTG AAA CTG GTG CGC TTC ATG CCT GCC CTG CGG 2916
Thr Phe Arg Leu Leu Arg Val Leu Lys Leu Val Arg Phe Met Pro Ala Leu Arg

CGC CAG CTC GTG GTG CTC ATG AAG ACC ATG GAC AAC GTG GCC ACC TTC TGC ATG 2970
Arg Gln Leu Val Val Leu Met Lys Thr Met Asp Asn Val Ala Thr Phe Cys Met

CTG CTC ATG CTC TTC ATC TTC ATC TTC AGC ATC CTT GGG ATG CAT ATT TTT GGC 3024
Leu Leu Met Leu Phe Ile Phe Ile Phe Ser Ile Leu Gly Met His Ile Phe Gly

TGC AAG TTC AGC CTC CGC ACG GAC ACT GGA GAC ACG GTG CCC GAC AGG AAG AAC 3078
Cys Lys Phe Ser Leu Arg Thr Asp Thr Gly Asp Thr Val Pro Asp Arg Lys Asn

TTC GAC TCC CTG CTG TGG GCC ATC GTC ACT GTG TTC CAG ATC CTC ACC CAG GAG 3132
Phe Asp Ser Leu Leu Trp Ala Ile Val Thr Val Phe Gln Ile Leu Thr Gln Glu

GAC TGG AAC GTC GTT CTC TAC AAT GGC ATG GCC TCC ACT TCT CCC TGG GCC TCC 3186
Asp Trp Asn Val Val Leu Tyr Asn Gly Met Ala Ser Thr Ser Pro Trp Ala Ser

CTC TAC TTT GTC GCC CTC ATG ACC TTC GGC AAC TAT GTG CTC TTC AAC CTG CTG 3240
Leu Tyr Phe Val Ala Leu Met Thr Phe Gly Asn Tyr Val Leu Phe Asn Leu Leu

GTG GCC ATC CTG GTG GAG GGC TTC CAG GCG GAG GTG ACT GTG GTC TTG GCA GAG 3294
Val Ala Ile Leu Val Glu Gly Phe Gln Ala Glu Val Thr Val Val Leu Ala Glu

GAA GCA CCC CCA CAG GGC CTG CGA AAG ACT GGG CGA GGG AGA GGT GGC CTG GAT 3348
Glu Ala Pro Pro Gln Gly Leu Arg Lys Thr Gly Arg Gly Arg Gly Gly Leu Asp

GGG GGA GGG CTG CAA TTC AAA CTT CTA GCA GGC AAC CTA TCC CTA AAG GAG GGG 3402
Gly Gly Gly Leu Gln Phe Lys Leu Leu Ala Gly Asn Leu Ser Leu Lys Glu Gly

GTT GCT GAT GAG GTG GGT GAC GCC AAT CGC TCC TAC TCG GAC GAG GAC CAG AGC 3456
Val Ala Asp Glu Val Gly Asp Ala Asn Arg Ser Tyr Ser Asp Glu Asp Gln Ser

TCA TCC AAC ATA GAA GAG TTT GAT AAG CTC CAG GAA GGC CTG GAC AGC AGC GGA 3510
Ser Ser Asn Ile Glu Glu Phe Asp Lys Leu Gln Glu Gly Leu Asp Ser Ser Gly

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GAT CCC AAG CTC TGC CCA ATC CCC ATG ACC CCC AAT GGG CAC CTG GAC CCC AGT  3564
Asp Pro Lys Leu Cys Pro Ile Pro Met Thr Pro Asn Gly His Leu Asp Pro Ser

CTC CCA CTG GGT GGG CAC CTA GGT CCT GCT GGG GCT GCG GGA CCT GCC CCC CGA  3618
Leu Pro Leu Gly Gly His Leu Gly Pro Ala Gly Ala Ala Gly Pro Ala Pro Arg

CTC TCA CTG CAG CCG GAC CCC ATG CTG GTG GCC CTG GGC TCC CGA AAG AGC AGC  3672
Leu Ser Leu Gln Pro Asp Pro Met Leu Val Ala Leu Gly Ser Arg Lys Ser Ser

GTC ATG TCT CTA GGG AGG ATG AGC TAT GAC CAG CGC TCC CTG GTG GGT GGT CTT  3726
Val Met Ser Leu Gly Arg Met Ser Tyr Asp Gln Arg Ser Leu Val Gly Gly Leu

AGA GCC ACA GCG GGG GTG CAG GCT GCC TTT GGG CAC CTG GTG CCC CAG CCG TGG  3780
Arg Ala Thr Ala Gly Val Gln Ala Ala Phe Gly His Leu Val Pro Gln Pro Trp

GTG TGC CTG TGG GGC GCT GAC CCG AAC GGG AAC TCC TTC CAG TCC AGC TCC CGG  3834
Val Cys Leu Trp Gly Ala Asp Pro Asn Gly Asn Ser Phe Gln Ser Ser Ser Arg

AGC TCC TAC TAC GGG CCA TGG GGC CGC AGC GCG GCC TGG GCC AGC CGT CGC TCC  3888
Ser Ser Tyr Tyr Gly Pro Trp Gly Arg Ser Ala Ala Trp Ala Ser Arg Arg Ser

AGC TGG AAC AGC CTC AAG CAC AAG CCG CCG TCG GCG GAG CAT GAG TCC CTG CTC  3942
Ser Trp Asn Ser Leu Lys His Lys Pro Pro Ser Ala Glu His Glu Ser Leu Leu

TCT GCG GAG CGC GGC GGC GGC GCC CGG GTC TGC GAG GTT GCC GCG GAC GAG GGG  3996
Ser Ala Glu Arg Gly Gly Gly Ala Arg Val Cys Glu Val Ala Ala Asp Glu Gly

CCG CCG CGG GCC GCA CCC CTG CAC ACC CCA CAC GCC CAC CAC GTT CAT CAC GGG  4050
Pro Pro Arg Ala Ala Pro Leu His Thr Pro His Ala His His Val His His Gly

CCC CAT CTG GCG CAC CGC CAC CGC CAC CAC CGC CGG ACG CTG TCC CTC GAC AAC  4104
Pro His Leu Ala His Arg His Arg His His Arg Arg Thr Leu Ser Leu Asp Asn

AGG GAC TCG GTG GAC CTG GCC GAG CTG GTG CCC GCG GTG GGC GCC CAC CCC CGG  4158
Arg Asp Ser Val Asp Leu Ala Glu Leu Val Pro Ala Val Gly Ala His Pro Arg

GCC GCC TGG AGG GCG GCA GGC CCG GCC CCC GGG CAT GAG GAC TGC AAT GGC AGG  4212
Ala Ala Trp Arg Ala Ala Gly Pro Ala Pro Gly His Glu Asp Cys Asn Gly Arg
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ATG CCC AGC ATC GCC AAA GAC GTC TTC ACC AAG ATG GGC GAC CGC GGG GAT CGC  4266
Met Pro Ser Ile Ala Lys Asp Val Phe Thr Lys Met Gly Asp Arg Gly Asp Arg

GGG GAG GAT GAG GAG GAA ATC GAC TAC GTG AGT GGG GGC GGG GCC GAA GGG GAC  4320
Gly Glu Asp Glu Glu Glu Ile Asp Tyr Val Ser Gly Gly Gly Ala Glu Gly Asp

CTG ACC CTG TGC TTC CGC GTC CGC AAG ATG ATC GAC GTC TAT AAG CCC GAC TGG  4374
Leu Thr Leu Cys Phe Arg Val Arg Lys Met Ile Asp Val Tyr Lys Pro Asp Trp

TGC GAG GTC CGC GAA GAC TGG TCT GTC TAC CTC TTC TCT CCC GAG AAC AGG CTC  4428
Cys Glu Val Arg Glu Asp Trp Ser Val Tyr Leu Phe Ser Pro Glu Asn Arg Leu

AGG GAT CTG GGC TGG GTA AGC CTC GAG TGC CAG GGA AAG GTG GGT GAC CTC GTG  4482
Arg Asp Leu Gly Trp Val Ser Leu Glu Cys Gln Gly Lys Val Gly Asp Leu Val

GTG TGG GTG TAT GGT CAG AGG AGG CAG CGC CAG ACC ATT ATT GCC CAC AAA CTC  4536
Val Trp Val Tyr Gly Gln Arg Arg Gln Arg Gln Thr Ile Ile Ala His Lys Leu

TTC GAC TAC GTC GTC CTG GCC TTC ATC TTT CTC AAC TGC ATC ACC ATC GCC CTG  4590
Phe Asp Tyr Val Val Leu Ala Phe Ile Phe Leu Asn Cys Ile Thr Ile Ala Leu

GAG CGG CCT CAG ATC GAG GCC GGC AGC ACC GAA CGC ATC TTT CTC ACC GTG TCC  4644
Glu Arg Pro Gln Ile Glu Ala Gly Ser Thr Glu Arg Ile Phe Leu Thr Val Ser

AAC TAC ATC TTC ACG GCC ATC TTC GTG GGC GAG ATG ACA TTG AAG GTA GTC TCG  4698
Asn Tyr Ile Phe Thr Ala Ile Phe Val Gly Glu Met Thr Leu Lys Val Val Ser

CTG GGC CTG TAC TTC GGC GAG CAG GCG TAC CTA CGC AGC AGC TGG AAC GTG CTG  4752
Leu Gly Leu Tyr Phe Gly Glu Gln Ala Tyr Leu Arg Ser Ser Trp Asn Val Leu

GAT GGC TTT CTT GTC TTC GTG TCC ATC ATC GAC ATC GTG GTG TCC CTG GCC TCA  4806
Asp Gly Phe Leu Val Phe Val Ser Ile Ile Asp Ile Val Val Ser Leu Ala Ser

GCC GGG GGA GCC AAG ATC TTG GGG GTC CTC CGA GTC TTG CGG CTC CTG CGC ACC  4860
Ala Gly Gly Ala Lys Ile Leu Gly Val Leu Arg Val Leu Arg Leu Leu Arg Thr

CTA CGC CCC CTG CGT GTC ATC AGC CGG GCG CCG GGC CTG AAG CTG GTG GTG GAG  4914
Leu Arg Pro Leu Arg Val Ile Ser Arg Ala Pro Gly Leu Lys Leu Val Val Glu

ACA CTC ATC TCC TCC CTC AAG CCC ATC GGC AAC ATC GTG CTC ATC TGC TGT GCC  4968
Thr Leu Ile Ser Ser Leu Lys Pro Ile Gly Asn Ile Val Leu Ile Cys Cys Ala
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TTC TTC ATC ATC TTT GGC ATC CTG GGA GTG CAG CTC TTC AAG GGC AAG TTC TAC  5022
Phe Phe Ile Ile Phe Gly Ile Leu Gly Val Gln Leu Phe Lys Gly Lys Phe Tyr

CAC TGT CTG GGC GTG GAC ACC CGC AAC ATC ACC AAC CGC TCG GAC TGC ATG GCC  5076
His Cys Leu Gly Val Asp Thr Arg Asn Ile Thr Asn Arg Ser Asp Cys Met Ala

GCC AAC TAC CGC TGG GTC CAT CAC AAA TAC AAC TTC GAC AAC CTG GGC CAG GCT  5130
Ala Asn Tyr Arg Trp Val His His Lys Tyr Asn Phe Asp Asn Leu Gly Gln Ala

CTG ATG TCC CTC TTT GTC CTG GCA TCC AAG GAT GGT TGG GTG AAC ATC ATG TAC  5185
Leu Met Ser Leu Phe Val Leu Ala Ser Lys Asp Gly Trp Val Asn Ile Met Tyr

AAT GGA CTG GAT GCT GTT GCT GTG GAC CAG CAG CCT GTG ACC AAC CAC AAC CCC  5238
Asn Gly Leu Asp Ala Val Ala Val Asp Gln Gln Pro Val Thr Asn His Asn Pro

TGG ATG CTG CTG TAC TTC ATC TCC TTC CTG CTC ATC GTC AGC TTC TTT GTG CTC  5292
Trp Met Leu Leu Tyr Phe Ile Ser Phe Leu Leu Ile Val Ser Phe Phe Val Leu

AAC ATG TTT GTG GGT GTC GTG GTG GAG AAC TTC CAC AAG TGC CGG CAG CAC CAG  5346
Asn Met Phe Val Gly Val Val Val Glu Asn Phe His Lys Cys Arg Gln His Gln

GAG GCT GAA GAG GCA CGG CGG CGT GAG GAG AAG CGG CTG CGG CGC CTG GAG AAG  5400
Glu Ala Glu Glu Ala Arg Arg Arg Glu Glu Lys Arg Leu Arg Arg Leu Glu Lys

AAG CGC CGG AAG GCC CAG CGG CTG CCC TAC TAT GCC ACC TAT TGT CAC ACC CGG  5454
Lys Arg Arg Lys Ala Gln Arg Leu Pro Tyr Tyr Ala Thr Tyr Cys His Thr Arg

CTG CTC ATC CAC TCC ATG TGC ACC AGC CAC TAC CTG GAC ATC TTC ATC ACC TTC  5508
Leu Leu Ile His Ser Met Cys Thr Ser His Tyr Leu Asp Ile Phe Ile Thr Phe

ATC ATC TGC CTC AAC GTG GTC ACC ATG TCC CTG GAG CAC TAC AAT CAG CCC ACG  5562
Ile Ile Cys Leu Asn Val Val Thr Met Ser Leu Glu His Tyr Asn Gln Pro Thr
```

(2) INFORMATION FOR SEQ ID NO: 19:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 567
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii)MOLECULE TYPE: other nucleic acid
(iii) HYPOTHETICAL: no
(iv) ANTI-SENSE: no
(vi) ORIGINAL SOURCE:
(A) ORGANISM: human
(ix) FEATURE: human alpha-I partial sequence
(xi)SEQUENCE DESCRIPTION: SEQ ID NO: 19:

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ATG CGG ATC CTG GTG AAC CTG CTC CTG GAC ACA CTG CCC ATG CTG GGG AAT GTC   54
Met Arg Ile Leu Val Asn Leu Leu Leu Asp Thr Leu Pro Met Leu Gly Asn Val

CTG CTG CTC TGC TTC TTT GTC TTC TTC ACC TTT GGC ATC ATA GGT GTG CAG CTC  108
Leu Leu Leu Cys Phe Phe Val Phe Phe Thr Phe Gly Ile Ile Gly Val Gln Leu

TGG GCG GGC CTG CTG CGT AAC CGC TGC TTC CTG GAG GAG AAC TTC ACC ATA CAA  162
Trp Ala Gly Leu Leu Arg Asn Arg Cys Phe Leu Glu Glu Asn Phe Thr Ile Gln

GGG GAT GTG GCC TTG CCC CCA TAC TAC CAG CCG GAG GAG GAT GAT GAG ATG CCC  216
Gly Asp Val Ala Leu Pro Pro Tyr Tyr Gln Pro Glu Glu Asp Asp Glu Met Pro

TTC ATC TGC TCC CTG TCG GGC GAC AAT GGG ATA ATG GGC TGC CAT GAG ATC CCC  270
Phe Ile Cys Ser Leu Ser Gly Asp Asn Gly Ile Met Gly Cys His Glu Ile Pro

CCG CTC AAG GAG CAG GGC CGT GAG TGC TGC CTG TCC AAG GAC GAC GTC TAC GAC  324
Pro Leu Lys Glu Gln Gly Arg Glu Cys Cys Leu Ser Lys Asp Asp Val Tyr Asp

TTT GGG GCG GGG CGC CAG GAC CTC AAT GCC AGC GGC CTC TGT GTC AAC TGG AAC  378
Phe Gly Ala Gly Arg Gln Asp Leu Asn Ala Ser Gly Leu Cys Val Asn Trp Asn

CGT TAC TAC AAT GTG TGC CGC ACG GGC AGC GCC AAC CCC CAC AAG GGT GCC ATC  432
Arg Tyr Tyr Asn Val Cys Arg Thr Gly Ser Ala Asn Pro His Lys Gly Ala Ile

AGC TTT GAC AAC ATC GGT TAT GCT TGG ATT GTC ATC TTC CAG GTG ATC ACT CTG  486
Ser Phe Asp Asn Ile Gly Tyr Ala Trp Ile Val Ile Phe Gln Val Ile Thr Leu

GAA GGC TGG GTG GCG ATC ATG TAC TAC GTG ATG GAT GCT CTC TCC TTC TAC AAC  540
Glu Gly Trp Val Ala Ile Met Tyr Tyr Val Met Asp Ala Leu Ser Phe Tyr Asn

TTC GTC TAC TTC ATC CTG CTT ATC ATA                                      567
Phe Val Tyr Phe Ile Leu Leu Ile Ile
```

(2) INFORMATION FOR SEQ ID NO: 20:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 567
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear
(ii)MOLECULE TYPE: other nucleic acid
(iii) HYPOTHETICAL: no
(iv) ANTI-SENSE: no
(vi) ORIGINAL SOURCE:
(A) ORGANISM: rat
(ix) FEATURE: rat alpha-I partial sequence
(xi)SEQUENCE DESCRIPTION: SEQ ID NO: 20:

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ATG CGG ATC CTG GTG AAC CTG CTG CTC GAC ACG CTG CCC ATG CTG GGG AAC GTG   54
Met Arg Ile Leu Val Asn Leu Leu Leu Asp Thr Leu Pro Met Leu Gly Asn Val

CTC CTG CTC TGT TTC TTC GTC TTC TTC ATC TTC GGC ATC ATT GGC GTG CAG CTC  108
Leu Leu Leu Cys Phe Phe Val Phe Phe Ile Phe Gly Ile Ile Gly Val Gln Leu

TGG GCA GGC CTG CTA CGG AAC CGC TGC TTC CTG GAA GAA AAC TTC ACC ATA CAA  162
Trp Ala Gly Leu Leu Arg Asn Arg Cys Phe Leu Glu Glu Asn Phe Thr Ile Gln

GGG GAT GTG GCC CTG CCC CCT TAT TAC CAA CCA GAG GAG GAT GAC GAG ATG CCC  216
Gly Asp Val Ala Leu Pro Pro Tyr Tyr Gln Pro Glu Glu Asp Asp Glu Met Pro

TTT ATC TGC TCC CTG ACT GGG GAC AAT GGC ATC ATG GGC TGC CAC GAG ATC CCC  270
Phe Ile Cys Ser Leu Thr Gly Asp Asn Gly Ile Met Gly Cys His Glu Ile Pro

CCA CTG AAG GAG CAG GGC CGG GAA TGC TGC CTG TCC AAA GAT GAT GTG TAT GAC  324
Pro Leu Lys Glu Gln Gly Arg Glu Cys Cys Leu Ser Lys Asp Asp Val Tyr Asp

TTC GGG GCG GGG CGC CAG GAC CTC AAC GCC AGC GGT CTG TGC GTC AAC TGG AAC  378
Phe Gly Ala Gly Arg Gln Asp Leu Asn Ala Ser Gly Leu Cys Val Asn Trp Asn

CGC TAC TAC AAC GTC TGC CGC ACG GGC AAC GCC AAC CCT CAC AAG GGC GCC ATC  432
Arg Tyr Tyr Asn Val Cys Arg Thr Gly Asn Ala Asn Pro His Lys Gly Ala Ile

AAC TTT GAC AAC ATT GGC TAT GCC TGG ATT GTG ATT TTC CAG GTG ATC ACT CTG  486
Asn Phe Asp Asn Ile Gly Tyr Ala Trp Ile Val Ile Phe Gln Val Ile Thr Leu
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GAA GGC TGG GTG GAG ATC ATG TAC TAT GTG ATG GAC GCA CAT TCT TTC TAC AAC  540
Glu Gly Trp Val Glu Ile Met Tyr Tyr Val Met Asp Ala His Ser Phe Tyr Asn

TTC ATC TAC TTC ATC CTG CTT ATC ATA                                     567
Phe Ile Tyr Phe Ile Leu Leu Ile Ile
```

CLAIMS

1. An isolated DNA fragment comprising a sequence of nucleotides that encodes a calcium channel, wherein the sequence of nucleotides is selected from sequences of nucleotides encoding a protein including the sequence of amino acids set forth in SEQ ID. No. 19, and sequences of nucleotides that hybridize under non-stringent conditions to DNA encoding a protein including the sequence set forth in SEQ ID No. 19.

2. The DNA fragment of Claim 1, wherein the sequence of nucleotides is selected from sequences of nucleotides encoding a protein including the sequence of amino acids set forth in SEQ ID. No. 18, and sequences of nucleotides that hybridize under non-stringent conditions to DNA encoding a protein including the sequence set forth in SEQ ID No. 18.

3. The DNA fragment of Claim 1 or 2, wherein the calcium channel is a human neuronal calcium channel.

4. An isolated DNA fragment comprising a sequence of nucleotides that encodes a human calcium channel subunit, wherein the sequence of nucleotides is selected from sequences of nucleotides including the sequence set forth in SEQ ID No. 17.

5. A vertebrate expression vector containing the DNA fragment of any of Claims 1 to 4.

6. A eukaryotic cell transiently or stably transformed with the vertebrate expression vector of Claim 5, said cell expressing the calcium channel encoded by the DNA fragment.

7. A eukaryotic cell transiently or stably transformed with a heterologous DNA fragment according to any of Claims 1 to 4, said cell expressing the calcium channel encoded by the DNA fragment.

8. The eukaryotic cell of claim 6 or 7, wherein the cell is further transformed with and expresses an $\alpha 2\delta$ or a β calcium channel subunit, or both.

9. A method for the production of the α-$_{II}$ protein of an animal cell calcium channel comprising, culturing the cell of Claim 6 or 7 under conditions whereby the DNA encoding the calcium channel subunit is expressed and the α-$_{II}$ subunit is produced.

10. A process for producing the eukaryotic cell that is transiently or stably transformed and expresses a calcium channel, comprising the step of introducing RNA or DNA having a sequence selected from among sequences that encode a protein including the sequence of amino acids set forth in SEQ ID. No. 19, and sequences of nucleotides that hybridize under non-stringent conditions to DNA encoding a protein including the sequence set forth in SEQ ID No. 19 and RNA or DNA encoding an $\alpha 2\delta$ or β calcium channel subunit into a cell.

11. A method of identifying compounds capable of acting as agonists or antagonists for the α-$_{II}$ calcium channel, comprising contacting a cell according to claim 6 or 7 with an agent to be tested, and evaluating the interaction, if any, between the agent to be tested and the calcium channel.

12. An isolated DNA fragment having the sequence given by SEQ ID No. 19.

13. A method for mapping the distribution of calcium channel subunits within a tissue sample comprising the steps of exposing the tissue to a reagent comprising a directly or indirectly detectable label coupled to a DNA fragment comprising a sequence selected from among those sequences given by SEQ ID Nos. 13-20, and detecting reagent that has bound to the tissue.

Figure 1 (Part I)

Query = C54D2.5 CE02562 CALCIUM CHANNEL ALPHA-1 SUBUNIT LG:6
Database: Non-redundant Database of GenBank EST Division
824,500 sequences; 302,742,428 total letters.

H55225 CHR220164 Homo sapiens genomic clone C22_207 5'.
Length = 168

Plus Strand HSPs:

Score = 136 (63.8 bits), Expect = 2.5e-10, P = 2.5e-10
Identities = 23/31 (74%), Positives = 29/31 (93%), Frame = +1

```
Query:   440 VISLEGWTDIMYYVQDAHSFWNWIYFVLLIV 470
             VI LEGW  IMYYV DAHSF N IYF  LLI
Sbjct:     1 VITLEGWVEIMYYVMDAHSFYNFIYFILLII 93
```

H55617 CHR220556 Homo sapiens genomic clone C22_757 5'.
Length = 98
Plus Strand HSPs:

Score = 102 (47.9 bits), Expect = 2.8e-05, P = 2.8e-05
Identities = 19/23 (82%), Positives = 23/23 (100%), Frame = +2

```
Query:   243 NINLTAIRTVRVLRPLRAVNRIP 265
             NINL AIRTVRVLRPL A NR P
Sbjct:    29 NINLSAIRTVRVLRPLKAINRVP 97
```

Figure 1 (Part II)

H55223 CHR220162 Homo sapiens genomic clone C22_204 5'.
Length = 94
Plus Strand HSPs:

Score = 87 (40.8 bits), Expect = 0.0039, P = 0.0039
Identities = 14/19 (73%), Positives = 18/19 (94%), Frame = +2

```
Query:   154 MAVIMINCVTLGMYRPCED 172
             M VI  NCVTLGMY PC D
Sbjct:     2 MLVILLNCVTLGMYQPCDD 58
```

H55544 CHR220483 Homo sapiens genomic clone C22_651 5'.
Length = 123
Plus Strand HSPs:

Score = 65 (30.5 bits), Expect = 3.8, P = 0.98
Identities = 12/23 (52%), Positives = 18/23 (78%), Frame = +1

```
Query:   246 LTAIRTVRVLRPLRAVNRIPSMR 268
                RT R LRPLRA  R   MR
Sbjct:    55 IKSLRTLRALRPLRALSRFEGMR 123
```

Figure 1 (Part III)

F07776| HSC2HD061 H. sapiens partial cDNA sequence; clone c-2hd06

Length = 343

Plus Strand HSPs:

Score = 100 (46.9 bits), Expect = 0.00057, P = 0.00057
Identities = 21/41 (51%), Positives = 31/41 (75%), Frame = +3

```
Query:  1480 PTIIRVMRVLRIARVLKLLKMAKGIRSLLDTVGEALPQVGN 1520
             PT+ RV+R+ RI R+L+L+K AKGIR+LL  +  +LP + N
Sbjct:    57 PTLXRVIRLARIGRILRLIKGAKGIRTLLFALMMSLPALFN 179
```

INTERNATIONAL SEARCH REPORT

Inte. ional Application No
PCT/CA 98/00173

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C12N15/12 C07K14/705 C12N5/10 C12Q1/68 G01N33/68

According to International Patent Classification(IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C07K

Documentation searched other than minimumdocumentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and. where practical. search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document. with indication, where appropriate. of the relevant passages	Relevant to claim No.
A	TROFATTER JA ET AL: "An expression-independent catalog of genes from human chromosome 22." GENOME RES, OCT 1995, 5 (3) P214-24. UNITED STATES, XP002069420 see the whole document	1,12
A	& EMBL database Accession number H55544 08-12-95 Trofatter j.a. et al. see the whole document	1,12
A	WO 95 04144 A (NEUREX CORP) 9 February 1995 cited in the application see claims 1-10	1,5,6, 9-12

☐ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

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"O" document referring to an oral disclosure. use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents. such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of theinternational search	Date of mailing of the international search report
25 June 1998	09/07/1998

Name and mailing address of the ISA	Authorized officer
European Patent Office. P B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040. Tx. 31 651 epo nl. Fax: (+31-70) 340-3016	Gurdjian, D

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

Information on patent family members

Inte ..ional Application No
PCT/CA 98/00173

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9504144 A	09-02-1995	EP 0778890 A	18-06-1997
		JP 9501051 T	04-02-1997

Form PCT/ISA/210 (patent family annex) (July 1992)